# Identification of blast resistance genes in IRRI-bred rice varieties by segregation analysis based on a differential system

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## Abstract

A differential system for rice blast disease consists of standard blast isolates (*Pyricularia oryzae* Cavara..) and differential rice varieties (*Oryza sativa* L.) with known resistance genes. Monogenic lines for blast resistance have been newly developed at the International Rice Research Institute (IRRI) as international differential varieties and differential blast isolates from the Philippines were selected. Using a differential system based on the gene-for-gene theory, blast resistance genes were estimated in the IRRI-bred elite rice varieties. At least seven kinds of resistance genes -- *Pi20*(t), *Pita*, *Pik** (one of the *Pik* allele genes except *Pik-s*), *Pik-s*, *Pib*, *Piz-t*, and *Pii* or *Pi3* -- were estimated in 42 rice varieties on the basis of reaction patterns to 14 standard blast isolates. The reaction patterns of the IRRI varieties were compared with those of the blast monogenic lines. To confirm this gene estimation, genetic analysis was done using segregating populations derived from crosses between the IRRI varieties and a susceptible Indica-type variety, CO 39. The $BC_1F_2$ populations (with CO 39 as recurrent parent) were segregated for reaction to the specific standard isolates. Furthermore, the estimated genes were confirmed by allelism test against the blast resistance monogenic lines. As a result of the segregation analysis of 10 of 42 IRRI-bred varieties, seven genes -- *Pi20*(t), *Pita*, *Pik**, *Pia*, *Pib*, *Pik-s* -- and *Piz-t*, were identified. Genes *Pia*, *Pib*, *Pik-s*, and *Piz-t* were not estimated by reaction patterns to the blast isolates but identified by genetic analysis in some varieties. The effectiveness of the differential system, which is based on conventional methods and which does not require advanced facilities, is discussed as a fundamental tool to provide essential information to develop breeding programs for blast resistance.

**Keywords:** rice (*Oryza sativa* L.), blast (*Pyricularia oryzae* Cavara ), differential system, genetic analysis, resistance, IRRI variety

## Introduction

Blast disease, caused by *Pyricularia oryzae* Cavara is a serious disease of rice (*Oryza sativa* L.) worldwide. The use of host resistance is one of the most economical and effective means of controlling blast disease. Resistance to blast disease is governed by a gene-for-gene relationship between resistance gene in the host and avirulence gene in the blast pathogen (Kiyosawa, 1972; Silue et. al., 1992). Genetic studies performed in this context led to the identification of many blast resistance genes. Genetic studies in Indica-type varieties have been limited because the extremely changeable virulence of the blast fungus (Ou, 1985; Bonman et al., 1986) and the presence of several resistance genes in Indica-type varieties' genetic background (Mackill et al.; 1985; Yu et al., 1987) made these studies complicated.

The limitation in genetic studies on blast resistance could also be attributed to a lack of suitable differential system for blast resistance genes in the tropics. The 12 Japonica-type differential varieties (DVs) for blast resistance were selected by Yamada et al. (1976) and Kiyosawa (1981), but those DVs are known to carry additional gene(s) showing resistance to tropical blast isolates, which masked the targeted gene's reaction (Noda et al., 1999). To address this limitation, Yanoria et al. (2000) tried to clarify the pathogenicities of blast isolates from the Philippines using DVs and lines. Several blast isolates with distinct pathogenicities were selected and studied in detail by Tsunematsu et al. (2000) using a set of monogenic lines that contains 23 kinds of single resistance genes with the genetic background of a Chi-

nese variety Lijiang-xin-tuan-heigu (LTH).

In South and Southeast Asia, rice varieties bred in IRRI are widely grown and account for more than 80% of total rice production in this region (Khush, 1990). And these IRRI-bred varieties have been used for breeding materials in many countries of Tropical region. Using the differential system consisting of newly developed monogenic lines and differential blast isolates from the Philippines, kinds of blast resistance genes in IRRI-bred varieties were identified. In this paper, methodologies of gene identification are explained following 3 steps with special emphasis on a case of IR64, which were distributed and cultivated the most widely and was still one of leading varieties in tropical region At first, kind of blast resistance gene was estimated by reaction patterns to differential blast isolates (I). To confirm the gene estimation, segregation analysis using backcross progenies with a susceptible variety (II) and allelism test with DVs were conducted (III).

## I. Gene estimation based on reaction pattern to differential blast isolates

### Materials and methods

1. Rice plant materials

A total of 42 IRRI-bred rice varieties were used for blast resistance gene estimation (Table 1). The widely grown varieties, IR8, IR24, IR36, IR64 and IR72 were included. Eight varieties, designated as PSBRc by the Philippine Seed Board, which are IRRI developed varieties, were also included.

Nine monogenic lines, IRBL20-IR24 (*Pi20*(t)), IRBLta-CP1 (*Pita*), IRBLk-K (*Pik*), IRBLks-F5 (*Piks*), IRBLa-A (*Pia*), IRBLb-B (*Pib*), IRBLzt-T (*Pizt*), IRBLi-F5 (*Pii*), and IRBL3-CP4 (*Pi3*) were tested for comparison.

2. Inoculation and evaluation of resistance

Pregerminated seeds of each variety or population were sown on soil in a 26 × 35 cm plastic tray. Ten grams of ammonium sulfate was applied to each tray as basal fertilizer and 1 g was added 1 wk before inoculation. In all cases, susceptible cultivars CO39 and LTH were included in each tray to check the success of inoculation and the virulence of blast isolates used.

Fourteen blast isolates with known avirulence (Yanoria et al., 2000, Telebanco-Yanoria et al. 2007) from the Philippines were used for inoculation. Seedlings at the four-leaf stage were sprayed with 40-50 ml spore suspension per tray adjusted to $10^5$ spores $ml^{-1}$. Trays were placed inside the wet jute sacks for 18-24 h, and transferred to an air-conditioned glasshouse room at 23±3℃/30±5℃ night and day temperature. Blast incidence on each seedling was examined 6-7 d after inoculation. The reaction was classified into 0-5 scales with slight modifications as described previously by Mackill and Bonman (1992). Seedlings rated 0-2 were considered resistant (R), 3 was moderate resistant (M), and those rated 4-5, susceptible (S).

3. Resistance gene estimation

Gene(s) were estimated by comparing reaction patterns to the differential blast isolates of IRRI varieties with those of blast monogenic lines (Table 2). Using the Philippine blast isolates, 4 alleles of *Pik* loci (*Pik*, *Pik-h*, *Pik-m*, and *Pik-p*) can not be differentiated because all the monogenic lines with these genes showed the same reaction pattern (Tsunematsu et al. 2000). Therefore, those alleles were designated tentatively as *Pik** in this paper.

Although total 14 differential isolates were involved in this study, reactions to 3 distinct isolates, M36-1-3-10-1, IK81-25, and PO6-6, were considered for grouping of varieties and primary gene estimation as a first step. As a second step, reaction patterns to the other 11 isolates were also taken into consideration for gene estimation (Table 2).

### Results and discussion

1. Gene estimation in IR64

IR64 was resistant to 12 (M36-1-3-10-1, IK81-25, IK81-3, BN111, V850256, M39-1-3-8-1, M64-1-3-9-1, M39-1-2-21-2, BN209, V850196, B90002, and C923-49) out of 14 standard blast isolates tested (Table 3). Among the 9 monogenic lines tested, 6 lines (IRBL20-IR24, IRBLta-K1, IRBLb-B, IRBLa-A, IRBLks-F5, and IRBLzt-T) were resistant to the blast isolates which were avirulent to IR64. These results suggested that 6 blast resistant genes (*Pi20*(t), *Pita*, *Pib*, *Pia*, *Pik-s*, and *Piz-t*) which were harbored in the respective monogenic lines were estimated to exist in the genetic background of IR64. Three genes, *Pi20*(t), *Pita*, and *Pib* showed relatively wide spectrum of resistance and covered the resistance of the other 3 genes, *Pia*, *Pik-s*, and *Piz-t*. Therefore, the latter 3 were not estimated directly but possible to be harbored in the genetic background.

The other 3 monogenic lines, IRBLk-Ka, IRBLi-F5, and IRBL3-CP4 were resistant to a blast isolate PO6-6 which was virulent to IR64 (Table 3). This suggested that 3 resistant genes, *Pik*, *Pii*, and *Pi3* were not harbored in the genetic background of IR64.

2. Gene estimation in IRRI-bred varieties

IRRI-bred varieties showed various patterns to the differential blast isolates. The reaction patterns of these varieties were compared with those of DVs (Table 4). These varieties were classified into 8 variety groups (VG) based on reaction patterns to three distinct blast isolates, M36-1-3-10-1, IK81-25, and PO6-6 (Table 4). These 3 isolates showed incompatibility with 3 resistance genes, *Pi20*(t) resistant to M36-1-3-10-1, *Pita* resistant to IK81-25, and *Pik* resistant to PO6-6. From these specific reactions, presence or absence of these 3 genes was estimated in the varieties for each VG.

**Table 1.** List of IRRI-bred rice varieties used for identifing the blast resistance genes

| Variety | Year of release | Ecosystem suited | Line designation | Cross combination |
|---|---|---|---|---|
| IR 5 | 1967 | Irrigated | IR5-47-2 | Peta/ tangkai Rotan |
| IR 8 | 1966 | Irrigated | IR8-288-2 | Pata/Dee-geo-woo-gen |
| IR20 | 1969 | Irrigated | IR523-E576 | IR262-24-3/TKM6 |
| IR22 | 1969 | Irrigated | IR579-160-2 | IR8/Tadukan |
| IR24 | 1971 | Irrigated | IR661-1-140-3 | IR8/IR127-2-2 |
| IR26 | 1973 | Irrigated | IR1541-102-7 | IR24/TKM6 |
| IR 28 | 1974 | Irrigated | IR2061-214-3-8-2 | IR833///IR1561-149-1//IR24*4/O. nivara |
| IR 29 | 1974 | Irrigated | IR2061-464-4-14-1 | IR833///IR1561-149-1//IR24*4/O. nivara |
| IR 30 | 1974 | Irrigated | IR2153-159-1-4 | IR1541-102-6-3//IR24*4/O. nivara |
| IR32 | 1975 | Irrigated | IR2070-747-6-3-2 | IR20*2/O.nivara//CR94-13 |
| IR34 | 1975 | Irrigated | IR2061-213-2-17 | IR833-6-2-1-1//IR1561-149-1//IR24*4/O.nivara |
| IR36 | 1976 | Irrigated | IR2071-625-1-252 | IR1561-228-1-2/IR737//CR94-13 |
| IR38 | 1976 | Irrigated | IR2070-423-2-5-6 | IR20*2/O.nivara//CR94-13 |
| IR 40 | 1977 | Irrigated | IR2070-414-3-9 | IR20*2/O.nivara//CR94-13 |
| IR42 | 1977 | Irrigated | IR2071-586-5-6-3 | IR1561-228-1-2/IR737//CR94-13 |
| IR 43 | 1978 | Irrigated | IR1529-430-3 | IR305-3-17-1-3/IR661-1-140-3 |
| IR 44 | 1978 | Irrigated | IR2863-38-1-2 | IR1529-680-3/CR94-13//IR480-5-9-3 |
| IR 45 | 1978 | Irrigated | IR2035-242-1 | IR1416-128-5/IR1364-37-3-1//IR1824-1 |
| IR 46 | 1978 | Irrigated | IR2058-78-1-3-2 | IR1416-128-5/IR1364-37-3-1//IR1366-120-3-1/IR1539-111 |
| IR 48 | 1979 | Irrigated | IR4570-83-3-3 | IR1702-74-3-2/IR1721-11-6-8-3//IR2055-481-2 |
| IR 50 | 1979 | Irrigated | IR9224-117-2-3-3-2 | IR2153-14-1-6-2/IR28//IR36 |
| IR 52 | 1980 | Irrigated | IR5853-118-5 | Nam Sa-gui 19/IR2071-88//IR2061-214-3-6-20 |
| IR 54 | 1980 | Irrigated | IR5853-162-1-2-3 | Nam Sa-gui 19/IR2071-88//IR2061-214-3-6-20 |
| IR 56 | 1982 | Irrigated | IR13429-109-2-2-1 | IR4432-53-33/PTB33//IR36 |
| IR 58 | 1983 | Irrigated | IR9752-71-3-2 | IR28/Kwang-chang-ai//IR36 |
| IR 60 | 1983 | Irrigated | IR13429-299-2-1-3 | IR4432-53-33/PTB33//IR36 |
| IR 62 | 1984 | Irrigated | IR13525-43-2-3-1-3-2 | PTB33/IR30//IR36 |
| IR 64 | 1985 | Irrigated | IR18348-36-3-3 | IR5657-33-2-1/IR2061-465-1-5-5 |
| IR 65 | 1985 | Irrigated | IR21015-196-3-1-3 | Batatais/IR36//IR52 |
| IR 66 | 1987 | Irrigated | IR32307-107-3-2-2 | IR13240-108-2-2-3/IR9129-209-2-2-2-1 |
| IR 68 | 1988 | Irrigated | IR28224-3-2-3-2 | IR19660-73-4/IR2415-90-4-3-2//IR54 |
| IR 70 | 1988 | Irrigated | IR28228-12-3-1-1-2 | IR19660-73-4/IR54//IR9828-36-3 |
| IR 72 | 1988 | Irrigated | IR35366-90-3-2-1-2 | IR19661-9-2-3/IR1576-199-3-3//IR9129-209-2-2-2-1 |
| IR74 | 1988 | Irrigated | IR32453-20-3-2-2 | IR19661-131-1-2/IR15795-199-3-3 |
| PSBRc 1 | 1990 | Upland | IR10147-113-5-1-1-5 | KN-1B-361-1-8-6/IR1750-F5B-3//BPI76*9/Dawn |
| PSBRc 2 | 1991 | Irrigated | IR32809-26-3-3 | IR4215-301-2-2-6/BG90-2//IR19661-131-1-2 |
| PSBRc 4 | 1991 | Irrigated | IR41985-111-3-2-2 | IR4547-4-1-2/IR1905-81-3-1//IR25621-94-3-2 |
| PSBRc 10 | 1992 | Irrigated | IR50404-57-2-2-3 | IR33021-39-2-2/IR32429-47-3-2-2 |
| PSBRc 18 | 1994 | Irrigated | IR51672-62-2-1-1-2-3 | IR24594-204-1-3-2-6-2/IR28222-9-2-2-2-2 |
| PSBRc 20 | 1994 | Irrigated | IR57301-195-3-3 | IR35293-125-3-2-3/IR32429-47-3-2-2/PSBRc4 |
| PSBRc 28 | 1995 | Irrigated | IR56381-139-2-2 | IR28239-94-2-3-6-2/IR64 |
| PSBRc 30 | 1995 | Irrigated | IR58099-41-2-3 | IR72/IR24632-34-2 |

IRRI (1995) and Peng and Khush (2003) were modified.

**Table 2.** Reaction pattern of monoginic lines to standard blast isolates from the Philippines

| Monogenic lines as differential varieties (resistance gene) | Standsrd differential blast isolates from the Philippines | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M36-1-3-10-1 | IK81-25 | PO6-6 | IK81-3 | BN111 | Ca89 | V850256 | M39-1-3-8-1 | M64-1-3-9-1 | M39-1-2-21-2 | BN209 | V850196 | B90002 | C923-49 |
| IRBL20-IR24 (*Pi20*(t)) | R | S | S | S | R | S | S | S | S | R | S | S | R | R |
| IRBLta-CP1 (*Pita*) | S | R | S | R | S | S | R | R | R-M | S | S | R | R | S |
| IRBLk-Ka (*Pik*) | S | S | R | R | R | R | R | S | S | S | R | R | R | R |
| IRBLks-F5 (*Pik-s*) | S | S | S | S | S | S | S | S | S | S | S | R | S | S |
| IRBLa-A (*Pia*) | S | S | S | S | S | S | S | S | S | S | S | S | R | R |
| IRBLb-B *(Pib*) | S | S | S | S | S | S | S | S | S | S | R-M | S | R | R |
| IRBLzt-T (*Piz-t*) | S | S | S | S | S | S | S | R | R | S | S | S | R | R |
| IRBLi-F5 (*Pii*) | S | R-M | R | R-M | R | S | S | M | S | R-M | S | R | S | S |
| IRBL3-CP4 (*Pi3*(t)) | S | R | R | M | R | S | S | R-M | S | R | S | R | S | S |

Monogenic lines were developed as a set of diffrential varieties by Tsunematsu et al. 2000
Standard diffential blast isolates from the Philippines were selected by Telebanco-Yanoria et al. 2008.

**Table 3.** Blast resistance gene estimation in IR64 based on the differential system

| Rice genotype (Resistance gene harbored in the genetic background) | | Standard differential blast isolates from the Philippines | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | M36-1-3-10-1 | IK81-25 | PO6-6 | IK81-3 | BN111 | Ca89 | V850256 | M39-1-3-8-1 | M64-1-3-9-1 | M39-1-2-21-2 | BN209 | V850196 | B90002 | C923-49 |
| IR64 | (*Pi20*(t), *Pita*, *Pib*)* (*Pia*, *Pik-s*, *Piz-t*)** | R | R | S | R | R | S | R | R | R | R | R | R | R | R |
| IRBL20-IR24 | (*Pi20*(t)) | R | S | S | S | R | S | S | S | S | R | S | S | R | R |
| IRBLta-K1 | (*Pita*) | S | R | S | R | S | S | R | R | R-M | S | S | R | R | S |
| IRBLb-B | (*Pib*) | S | S | S | S | S | S | S | S | S | S | R-M | S | R | R |
| IRBLa-A | (*Pia*) | S | S | S | S | S | S | S | S | S | S | S | S | R | R |
| IRBLks-F5 | (*Pik-s*) | S | S | S | S | S | S | S | S | S | S | S | R | S | S |
| IRBLzt-T | (*Piz-t*) | S | S | S | S | S | S | S | R | R | S | S | S | R | R |
| IRBLk-Ka | (*Pik*) | S | S | R | R | R | R | R | S | S | S | R | R | R | R |
| IRBLi-F5 | (*Pii*) | S | R-M | R | R-M | R | S | S | M | S | R-M | S | R | S | S |
| IRBL3-CP4 | (*Pi3*(t)) | S | R | R | M | R | S | S | R-M | S | R | S | R | S | S |

S; susceptible, M; moderate resistant, and R; resistant.
*Estmated resistatce genes harboried in the IR64 genetic background, based on the reaction patterns to standard differential blast isolates from the Philippines
** Possible resistance gene harbored in the genetic background. These reaction of resistance genes are masked by the estimated genes which show the wide spectrums to the blast isolates
Three genes, *Pik*, *Pii*, and *Pi3*(t)are not harbored in the genetic background of IR64. Reaction patterns of the monogenic lines do not agree with that of IR64.

**Table 4.** Variety classification based on reaction pattern to 3 selected blast isolates

| Variety Group and differential variety | Reaction to standard blast isolate[1)] | | | Estimated gene(s) harboring in the genetic background | | |
|---|---|---|---|---|---|---|
| | M36-1-3-10-1 | IK81-25 | PO6-6 | | | |
| IRBL20-IR24 | R | S | S | *Pi20*(t) | - | - |
| IRBLta-CP1 | S | R | S | - | *Pita* | - |
| IRBLk-K | S | S | R | - | - | *Pik* |
| VG1 | S | S | S | - | - | - |
| VG2 | R | S | S | *Pi20*(t) | - | - |
| VG3 | S | R | S | - | *Pita* | - |
| VG4 | M | S | R | - | - | *Pik* |
| VG5 | R | S | R | *Pi20*(t) | - | *Pik-* |
| VG6 | S | R | R | - | *Pita* | *Pik* |
| VG7 | R | R | S | *Pi20*(t) | *Pita* | - |
| VG8 | R | R | R | *Pi20(*t) | *Pita* | *Pik* |

S; susceptible, M; moderate resistant, and R; resistant.

1) Blast isolates were selcted from the Philippines by Telebanco-Yanoria et al. 2008

Five IRRI varieties, IR20, IR28, IR30, IR45, and IR66, were susceptible to all 3 selected isolates and classified as VG1a (Table 4). None of the *Pi20*(t), Pita, or *Pik** were estimated in these varieties. These five varieties showed resistance to 4 isolates, BN209, V850196, B90002, and C923-49 (Table 5). These results can be explained by presence of *Pib* and *Pik-s* in VG1 (Table 2 and 5). Two varieties, IR29 and IR34 in VG1b showed resistance to 4 more isolates, IK81-25, IK81-3, M39-1-3-8-1, and M64-1-3-9-1. The resistant reactions to M39-1-3-8-1, and M64-1-3-9-1 were attributed to the presence of *Piz-t* in these 2 varieties, while those to the other 2 isolates, IK81-25 and IK81-3 were to unknown gene(s). From these reaction patterns, VG1 was further classified into 2 subgroups; i.e. VG1a (IR20, IR28, IR30, IR45, and IR66 estimated with *Pib* and *Pik-s*) and VG1b (IR29 and IR34 with *Pib*, *Pik-s*, *Piz-t* and unknown gene).

Seven varieties, IR8, IR22, IR24, IR26, IR43, PSBRc2, and PSBRc30, showed resistance to a blast isolate M36-1-3-10-1 and were classified as VG2 (Table 4). VG2 was estimated to harbor *Pi20*(t), because this gene is incompatible with the isolate M36-1-3-10. Resistance genes, *Pita* or *Pik** were thought to be absent in VG2, because these varieties was susceptible to both IK81-25 and PO6-6 which are avirulent to these genes. All 7 varieties in this group also showed resistance to 3 isolates, BN209, B90002, and C923-49, which were avirulent to *Pib* (Table 2 and 5). These results suggested that varieties in VG2 were likely to harbor *Pi20*(t) and *Pib*.

Furthermore, VG2 was divided into 3 subgroups, VG2a, VG2b, and VG2c based on differences in reaction to 3 isolates, M39-1-3-8-1, M64-1-3-9-1, and V850196 (Table 5). The varieties in subgroups, VG2a and VG2c showed resistance to V850196 whereas VG2b showed moderate resistance. Resistance to this isolated was estimated to be controlled by a resistance gene *Pik-s*, because *Pita* nor *Pik** was not estimated in VG2. The moderate resistance to this isolate of VG2b containing only one variety IR43 could be attributed to the presence of an unknown gene. VG2b was resistant to M39-1-3-8-1 and moderately resistant to M64-1-3-9-1. This reaction pattern could not be explained by resistance gene in the DVs used in this study. VG2c which included only one variety, PSBRc2, was resistant to M39-1-3-8-1 and M64-1-3-9-1 likely because of the presence of *Piz-t*.

Among the IRRI varieties tested, many of them were resistant to IK81-25 and susceptible to both M36-1-3-10 and PO6-6 (Table 4). From this reaction pattern, 17 varieties, IR5, IR32, IR36, IR38, IR40, IR42, IR44, IR50, IR52, IR54, IR58, IR60, IR62, IR65, IR68, IR72, and PSBRc4, were classified as VG3 and considered to harbor *Pita* and not to harbor *Pi20*(t) or *Pik**. Resistance to IK81-3, V850256, M39-1-3-8-1, M64-1-3-9-1, V850196, and B90002 were considered to be attribution of *Pita* (Table 2 and 5). VG3 varieties were also resistant to blast isolates BN209 and C923-49 which were avirulent to *Pib*. Therefore, VG3 were estimated to harbor *Pita* and *Pib*. The presence of *Pita* and *Pib* did not contradicted to susceptible reaction to PO6-6, M36-1-3-10-1, BN111, Ca89, and M39-1-2-21-2.

**Table 5.** Summary of estimated resistance gene in IRRI-bred ricevarieties using standard differential blast isolates from the Philippines

| Variety Group | Monogenic lines and IRRI-bred varieties | Standard differential blast isolate | | | | | | | | | | | | | | Resistance genes estimated and harboring in the genetic background | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | M36-1-3-10-1 | IK81-25 | PO6-6 | IK81-3 | BN111 | Ca89 | V850256 | M39-1-3-8-1 | M64-1-3-9-1 | M39-1-2-21-2 | BN209 | V850196 | B90002 | C923-49 | | | | | | | |
| | IRBL20-IR24 | R | S | S | S | R | S | S | S | S | R | S | S | R | R | *Pi20*(t) | - | - | - | - | - | - |
| | IRBLta-CP1 | S | R | S | R | S | S | R | R | R | S | S | R | R-M | M | - | *Pita* | - | - | - | - | - |
| | IRBLk-K | S | S | R | S | S | S | S | S | S | S | R-M | S | R | R | - | - | *Pik* | - | - | - | - |
| VG1a | IR20, IR28, IR30, IR45, IR66 | S | S | S | S | S | S | S | S | S | S | R | R | R | R | - | - | - | *Pik-s* | *Pib* | - | - |
| VG1b | IR29, IR34 | S | R-M | S | R-M | S | S | S | R | R | S | R | R | R | R | - | - | - | *Pik-s* | *Pib* | *Piz-t* | - |
| VG2a | IR8, IR22, IR24, IR26, PSBRc30 | R | S | S | S | R | S | S | S | S | R | R | R | R | R | *Pi20*(t) | - | - | | *Pib* | - | - |
| VG2b | IR43 | R | S | S | S | R | S | S | R | M | R | R | M | R | R | *Pi20*(t) | - | - | | *Pib* | | *Pix* |
| VG2c | PSBRc2 | R | S | S | S | R | S | S | R | R | R | R | R | R | R | *Pi20*(t) | - | - | | *Pib* | *Piz-t* | |
| VG3 | IR5, IR32, IR36, IR38, IR40, IR42, IR44, IR50, IR52, IR54, IR58, IR60, IR62, IR65, IR68, IR72, PSBRc4 | S | R | S | R | S | S | M | R | R | S | R | R | R | R | - | *Pita* | | | *Pib* | - | - |
| VG4 | PSBRc1 | M | S | R | M | R | R | R | S | S | S | R | R | R | R | | - | *Pik** | | - | - | *Pix* |
| VG5 | IR74 | R | S | R | R | R | R | R | S | S | R | R | R | R | R | *Pi20*(t) | - | *Pik** | | - | - | - |
| VG6 | IR56, IR70 | S | R | R | R | R | R | R | R | R | S | R | R | R | R | | *Pita* | *Pik** | | - | - | - |
| VG7a | IR46, IR48, IR64, PSBRc28 | R | R | S | R | R | S | R | R | R | R | R | R | R | R | *Pi20*(t) | *Pita* | - | | *Pib* | - | - |
| VG7b | PSBRc10, PSBRc18, PSBRc20 | R | R | R | R | R | S | R | R | R | R | R | R | R | R | *Pi20*(t) | *Pita* | - | | *Pib* | - | *Pii* or *Pi3*(t) |

S; susceptible, M; moderate resistant, and R; resistant.
*Pik**: One of the allele of *Pik* gene expect for *Pik-s*.
These standard differential blast isolates from the Philippines can not classify the multiple alleles of *Pik*; *Pik*, *Pik-h*, *Pik-m*, and *Pik-p*.
*Pix*: Unknow gene

Only one variety, PSBRc1 was resistant to PO6-6, susceptible to IK81-25, and moderately resistant to M36-1-3-10. This variety was classified as VG4 and estimated to harbor *Pik** but not *Pita* (Table 4). *Pi20*(t) was thought to be absent in PSBRc1, because this variety was susceptible to M39-1-2-21-2 which is incompatible with the gene (Table 2 and 5). The moderate resistance of this variety to M36-1-3-10 was probably conferred by unknown gene(s) other than genes in DVs tested.

IR74 was susceptible to only 1 isolate IK81-25 among the 3 isolate for primarily estimation of 3 genes. This variety was classified as VG5 and estimated to harbor *Pi20*(t) and *Pik** among the 3 genes (Table 4). The presence of these genes did not contradict to the susceptible reaction to M39-1-8-1 and M64-1-3-9-1 of this variety (Table 5).

Two varieties, IR56 and IR70 were resistant to PO6-6 and IK81-25 and susceptible to M36-1-3-10-1 (Table 4). These varieties were classified as VG6 and estimated to harbor *Pita* and *Pik** among the 3 selected genes for primary estimation. The reaction pattern of VG6 was corresponded with that of additional effect pattern of IRBLta-CP1 with *Pita* and IRBLk-Ka with *Pik* (Table 2 and 5).

Four varieties, IR46, IR48, IR64, and PSBRc28, were resistant to 2 isolates, M36-1-3-10-1 and IK81-25, and estimated to have *Pi20*(t) and *Pita* by primary estimation and classified as VG7 (Table 4). Three varieties, PSBRc10, PSBRc18, and PSBRc20 were resistant to PO6-6 in addition to those 2 isolates. These 3 varieties were estimated to have *Pi20*(t) and *Pita* but not *Pik**, because these varieties were susceptible to Ca89 which was avirulent to *Pik** (Table 5). These 3 varieties were classified as VG7b, while the former 4 were classified as VG7a. These 7 varieties were resistant to BN209 which was avirulent to *Pik** and *Pib* (Table 2 and 5). *Pik** was not considered to be harbored in these varieties as mentioned earlier. Therefore, *Pib* was estimated in these varieties in addition to *Pi20*(t) and *Pita*. Resistance to the blast isolate, PO6-6 in varieties classified as VG7b, was estimated to be due to the presence of *Pii* or *Pi3*.

## II. Segregation analyses using backcross populations

### Materials and methods

1. Rice plant materials

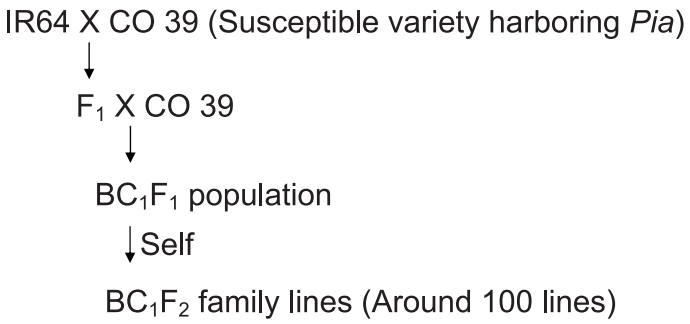
As shown in table 3, resistance to a certain blast isolate is governed by one or more genes in a variety. Segregation analysis using the $BC_1F_2$ family lines was conducted to determine the number of genes conferring resistance to the specific blast isolate. Six IRRI varieties representing several VGs, IR34 (VG1b), IR36 and IR60 (VG3), IR74 (VG5), and IR46 and IR64 (VG7a), were crossed with a susceptible check variety CO39 (a case of IR64 is shown in Fig. 1). The $F_1$ plants were backcrossed with CO39 as the recurrent parent to generate $BC_1F_1$. The $BC_1F_2$ family lines with population sizes ranging from 53 to 104 were generated by selfing and used for the segregation analysis.

2. Inoculation and evaluation of resistance

The same procedure described above was employed for blast inoculation test. Differential blast isolates from the Philippines with known pathogenecity were used for the inoculation test to identify resistance genes. Each line consisting of 20 seedlings was inoculated with selected blast isolates avirulent to the expected genes.

3. Estimation of number of resistance genes

Following the procedure proposed by Toriyama et al. (1986), the number of genes conferring resistance in a variety was estimated from the segregation of $BC_1F_2$ lines to each blast isolate. Three ratios, 1:1, 3:1, and 7:1, for segregating for resistance (heterozygote) against susceptible (homozygote) lines were expected for one, two, and three genes controlling resistance, respectively (Fig. 2, Fig. 3, and Fig. 4, respectively). For example as shown in Fig. 2, where a single dominant gene in target rice




**Appendix 1.** Hybrid population for segregation analysis of resistance genes in the IRRI bred variety IR64

The hybrid population was developed by back crossing between IR64 and the susceptible variety CO 39 as the recurrent parent.

variety confers the resistance, ratio of number of resistant against susceptible $BC_1F_1$ plants and that of segregating against susceptible $BC_1F_2$ family lines are 1:1.

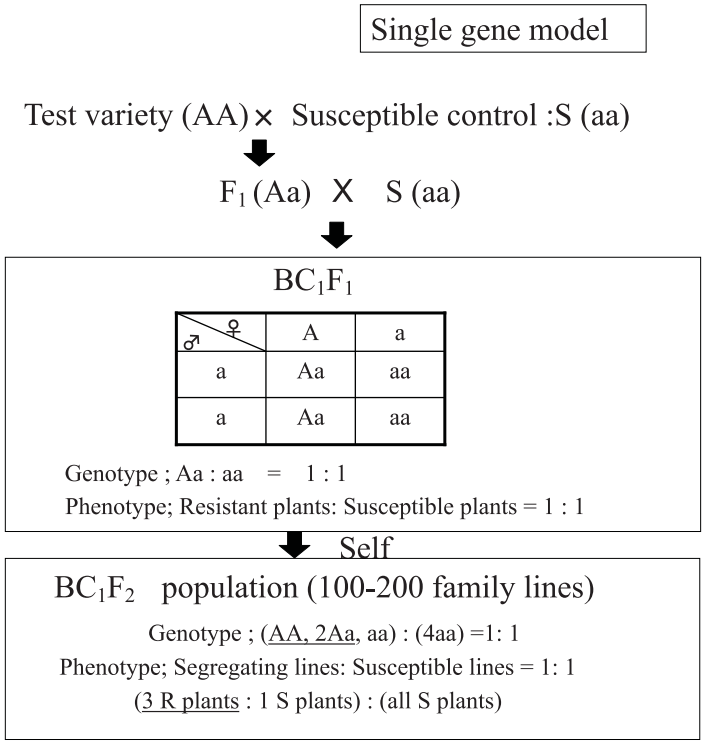
Segregation analysis using $F_2$ or $F_3$ family lines are also possible for estimation of number of resistance genes, although it was not employed in this study. The expected ratios for all resistant plants : segregation : all susceptible plants are 1:2:1 and 7:8:1 in $F_3$ family lines for a single and two dominant gene(s) control, respectively (Fig. 5 and 6, respectively).

**Results and discussion**

1. Estimation of number of resistance genes in IR64

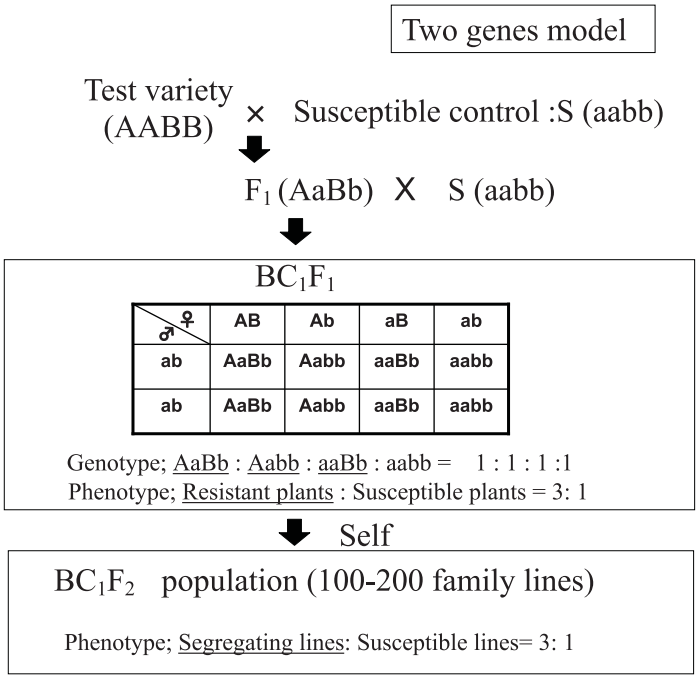
In IR64 of VG7a, 72 $BC_1F_2$ lines were generated by backcross with CO39 (Fig. 1). These lines were tested against four isolates, BN111, IK81-25, M64-1-3-9-1, and BN209 (Table 6). The ratios of number of all resistant lines (resistant homozygote) : segregating lines (heterozygote) : all susceptible lines (susceptible homozygote) in response to two isolates, BN111 and IK81-25 were well fit to 0:1:1 ($\chi^2$=0.06, $p$=0.81, and $\chi^2$=1.39, $p$=0.24, respectively). These results suggested that the resistance to each of these 2 isolates was controlled by a single dominant gene. The ratios in number of lines of all resistant : segregating : all susceptible to the remaining two isolates, M64-1-3-9-1 and BN209 were well fit to 0:3:1 ($\chi^2$=0.67, $p$=0.41 and $\chi^2$=0.08, $p$=0.78, respectively). From these results, it was estimated that the resistance to each of these 2 isolates was controlled by 2 dominant genes.

Isolate BN111 is avirulent to *Pi20*(t) and *Pik** (Table 3). A single dominant gene controlling resistance of IR64 to this isolate was estimated to be *Pi20*(t) but not *Pik**, because the gene *Pik** was not estimated in IR64 in the previous estimation by differential system (Table 5 and 6). Similarly, a single dominant gene for resistance to IK81-25 was estimated to be *Pita* (Table 3 and 6). The remaining 2 blast isolates, M64-1-3-9-1 and BN209 which were estimated to be controlled by 2 genes, were avilurent to *Pita* and *Piz-t*, and to *Pib*, respectively (Table 3). From these results, four genes (*Pi20*(t), *Pita*, *Piz-t*, and *Pib*) were detected in IR64 to control the segregation for resistance to the isolates used. However, one of the two genes could not be identified in the segregation analysis using BN209.



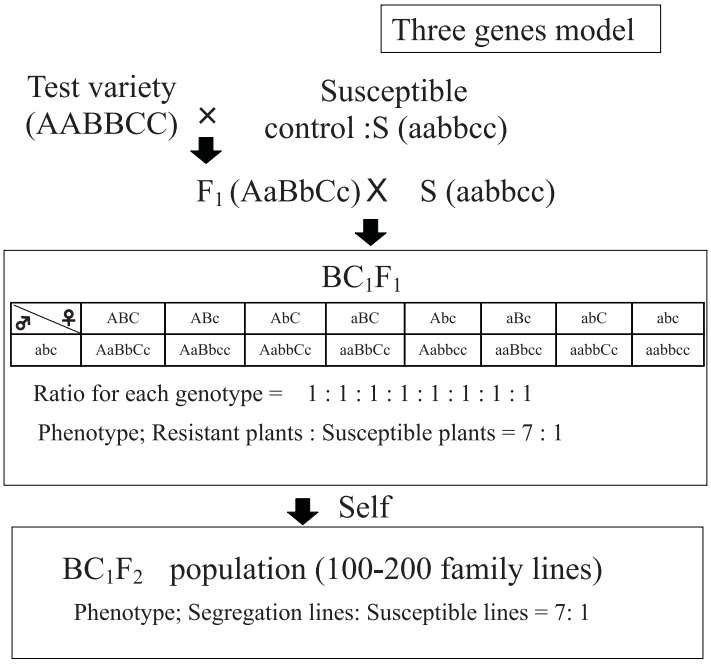

**Appendix 2.** Segregation analysis for blast resistance gene using $BC_1F_2$ plants or $BC_1F_2$ family lines – single gene model

A; resistance gene harbored in the genetic background. Where a single dominant gene in a test rice variety confers resistance, the ratio of number of resistant to susceptible $BC_1F_1$ plants and that of segregating to susceptible $BC_1F_2$ family lines is 1 : 1.
Avirulence blast pathogen for the resistance gene, A, is used in segregation analysis. Avirulence pathogen is selected by prior inoculation test on the test variety. When the virulent blast isolate is used, all progeny will be susceptible.



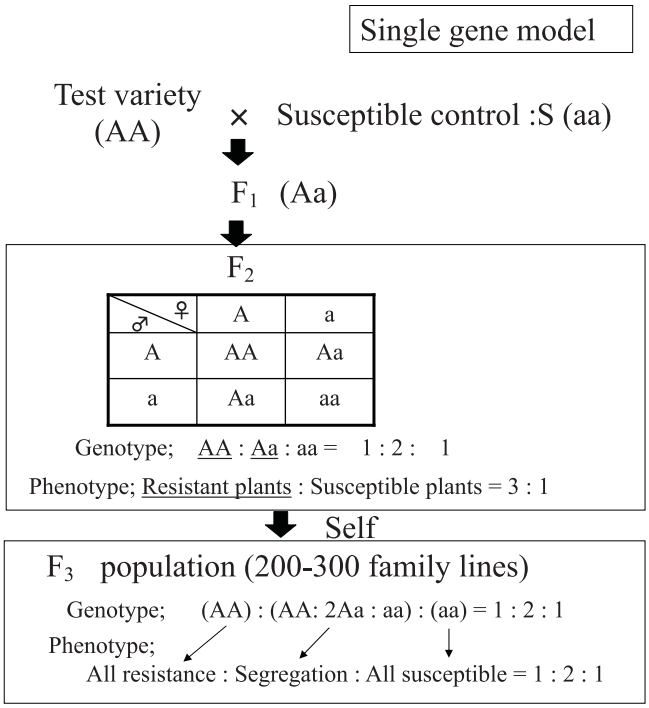

**Appendix 3.** Segregation analysis for blast resistance gene using $BC_1F_2$ plants or $BC_1F_2$ family lines – two gene model

A, B; Resistance genes harbored in the genetic background.
Where two dominant genes of test rice variety confer resistance independently, the segregation ratio in the $BC_1F_1$ plants for resistant (heterozygote) and susceptible (homozygote) plants is 3:1. Accordingly, the ratio of number of $BC_1F_2$ family lines segregating for resistance to all susceptible lines is 3:1.
A blast isolate avirulent to the differential variety with a known resistance gene is used for the inoculation test.



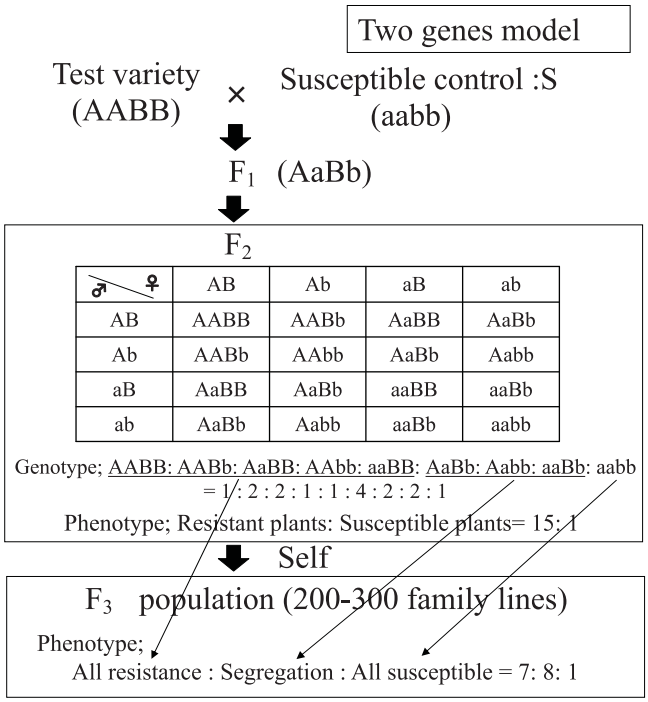

**Appendix 4.** Segregation analysis for blast resistance gene using $BC_1F_2$ plants or $BC_1F_2$ family lines – three gene model

A, B, C; Resistance genes harbored in the background.
Where three dominant genes in the genetic background of test rice variety confer resistance independently, the segregation ratio in the $BC_1F_1$ plants for resistant (heterozygote) and susceptible (homozygote) plants is 7:1. Accordingly, the ratio of number of $BC_1F_2$ family lines with segregating for resistance to all susceptible lines is 7:1.
A blast isolate avirulent to the differential variety with known resistance gene is used for the inoculation test.




**Appendix 5.** Segregation analysis for blast resistance genes using $F_2$ plants or $F_3$ family lines – single gene model

A; Resistance gene harbored in the genetic background. Where a single dominant gene in the genetic background of a test rice variety confers resistance, the ratio for the number of resistant homozygote : heterozygote : susceptible homozygote $F_2$ plants is 1:2:1. Accordingly, the ratio for number of $F_3$ family lines is 1(all R): 2 (segregation): 1 (all S). A blast isolate avirulent to the differential variety with the known resistance gene is used for the inoculation test.




**Appendix 6.** Segregation analysis for a blast resistance gene using $F_2$ plants or $F_3$ family lines – two gene model

A, B; Resistance genes harbored in the genetic background. Where two dominant genes in the genetic background of a test rice variety confer resistance independently, the ratio for the number of resistant homozygotes with at least one gene: heterozygote : susceptible homozygote $F_2$ plants is 7:8:1. Accordingly, the ratio for the number of $F_3$ family lines is 7 (all R): 8 (segregation) : 1 (all S). A blast isolate avirulent to the differential variety with the known resistance gene is used for the inoculation test.

2. Estimation of number of resistance genes in IRRI-bred varieties

Table 7 shows the results of the segregation analyses in six $BC_1F_2$ populations. In IR34 (VG1b), three genes, *Pik-s*, *Pib*, and *Piz-t*, were previously estimated (Table 5), hence, genetic analysis was performed to confirm these genes. A total of 104 $BC_1F_2$ family lines were examined for segregation to five isolates, M39-1-3-8-1, M64-1-3-9-1, V850196, BN209, and B90002. Against M39-1-3-8-1 and M64-1-3-9-1, 48 lines were segregating resistant and 56 lines were susceptible homozygote, and a co-segregation for resistance was recognized between these isolates. The segregation fit a 1:1 ratio expected of a single dominant gene control. Since both isolates were avirulent to *Piz-t* and *Pita*, but IR34 in VG1 was previously estimated not to have *Pita*, the segregation for resistance was therefore governed by *Piz-t*. To isolates V850196 and BN209, the segregation rates between segregating resistant and susceptible homozygote lines fit a 3:1 ratio, suggesting that two dominant genes controlled the resistance to each isolate. One of the genes was identified as *Pik-s* to V850196 based on the estimation by reaction patterns, but the other one could not be identified by the differential system. Similarly, *Pib* was identified as one of the resistance genes to BN209 but the other one was not identified. The same lines were also examined for *Pia*, a resistance gene that was not estimated by reaction patterns because *Pia* was masked by *Pi20*(t), *Pita*, or *Pik**. All the lines showed a resistant reaction to isolate B90002, which was avirulent to *Pia*. Therefore, *Pia* was identified in IR34 since it was known to be included in the recurrent parent, CO39 (Tsunematsu et al., 2000). On the basis of these results, the presence of *Pib*, *Pik-s*, and *Piz-t* was confirmed in IR34, while another gene *Pia* and at least an unknown one were also detected.

Two varieties from VG 3, IR36 and IR60, previously estimated to harbor *Pita* and *Pib*, were analyzed (Table 7). Results showed that *Pita* could be identified in IR36 using isolates IK81-3, and V850196 which are avirulent to *Pita*. The 61 $BC_1F_2$ lines derived from a cross between IR36 and CO39 exhibited single gene segregation to IK81-3. The segregation for resistance was due to *Pita*. To isolates V850196, the segregation rates between segregating resistant and susceptible homozygote lines fit a 3:1 ratio, suggesting that two dominant genes controlled the resistance. Therefore, *Pib*, which was previously estimated and incompatible to this isolate, was identified in IR36 as one of the gene conferring the resistant to this isolate. *Pik-s* was also identified in IR36 as another resistance gene to V850196 with considering the reaction pattern (Table 5). Similarly, the resistance to BN209 that has avirulence to *Pib* and *Pik** among the gene tested, was considered to be controlled by 2 dominant genes. Previous analysis showed that *Pik** was not included in VG 3; hence, segregation for resistance to BN209 was related to *Pib* and another unknown gene. As a result of segregation analysis, 3 genes, *Pib*, *Pita* and *Pik-s* as well as one unknown gene were confirmed to be harbored in IR36.

The presence of two genes, *Pita* and *Pib*, was analyzed in IR60 of VG3. Using 53 $BC_1F_2$ lines, a digenic segregation to each of the three isolates, IK81-3, M64-1-3-9-1, and V850196 was obtained, and three genes controlled were estimated for resistance to BN209 (Table 7). IK81-3 is avirulent to *Pita* and *Pik**, while the latter was not present in VG3 by the previous estimation. M64-1-3-9-1 was avirulent to *Pita* and *Piz-t*, V850196 to *Pita* and *Pik-s*, and BN209 to *Pib* and *Pik** in the differential system. Thus, 3 genes, *Pita*, *Pik-s*, and *Pib*, were considered to be related in the segregation. However, the other one gene for resistance to IK81-3 and two genes for resistance to BN209 could not be identified by reaction patterns of the differential system.

IR74 in VG5 was estimated to harbor 2 genes, *Pi20*(t) and *Pik** by reaction patterns (Table 5). This variety was

**Table 6.** Segregation for resistance in $BC_1F_2$ populations derived from a cross between IR64 and a susceptible variety CO 39 as the recurrent parent

| Differntial blast isolate | Reaction of monogenic line | | | | No. of $BC_1F_2$ lines | | | | $\chi^2$ value | | *P* (df =1) | Estimated no. of resistance gene | Estimated gene |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IRBL20-IR24 (*Pi20*) | IRBLta-K1(*Pita*) | IRBLb-B (*Pib*) | IRBLzt-T (*Piz-t*) | All resistant plants | Segregating | All susceptible | Total | 1:1 | 3:1 | | | |
| BN111 | R | S | S | S | 0 | 37 | 35 | 72 | 0.06 | - | 0.81 | 1 | *Pi20*(t) |
| IK81-25 | S | R | S | S | 0 | 41 | 31 | 72 | 1.39 | - | 0.24 | 1 | *Pita* |
| M64-1-3-9-1 | S | R | S | R | 0 | 51 | 21 | 72 | - | 0.67 | 0.41 | 2 | *Pita*, *Piz-t* |
| BN209 | S | S | R-M | S | 0 | 52 | 17 | 69 | - | 0.08 | 0.78 | 2 | *Pib*, one unknown |

analyzed using 76 $BC_1F_2$ lines for segregation to six isolates: PO6-6 and Ca89 for identification of *Pik**, M36-1-3-10-1 for *Pi20*(t), BN111 for *Pi20*(t) and *Pik**, BN209 for *Pib* and *Pik**, and B90002 for *Pia*, *Pik**, *Pi20*(t), *Pib*, and *Piz-t* (Table 7). In the segregation analysis on $BC_1F_2$ lines, resistance was controlled by a single dominant gene judging from the segregation ratio of 1:1 involving three isolates, PO6-6, Ca89, and M36-1-3-10-1. A co-segregation was also observed between isolates PO6-6 and Ca89. *Pik** was identified to control the segregation for PO6-6 and Ca89, and *Pi20*(t) was for isolate M36-1-3-10-1. A digenic segregation to isolates BN111 and BN209 were observed. One of the genes, *Pik**, was identified to be common in the segregation to both isolates.

**Table 7.** Segregation for resistance in $BC_1F_2$ populations derived from the crosses between IRRI-bred varieties and a susceptible variety CO 39 as the recurrent parent

| Variety (VG) | Isolate | No. of lines | | | | $\chi^2$ value[1)] | | | *P* (df=1) | Estimated no. of resistance gene | Estimated gene[2)] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | All resistant | Segregating resistant | All susceptible | Total | 1:1 | 3:1 | 7:1 | | | |
| IR34 (VG1b) | M39-1-3-8-1 | - | 48 | 56 | 104 | 0.62[a] | - | - | 0.43 | 1 | ***Piz-t*** |
| | M64-1-3-9-1 | - | 48 | 56 | 104 | 0.62[a] | - | - | 0.43 | 1 | ***Piz-t*** |
| | V850196 | - | 77 | 27 | 104 | - | 0.02 | - | 0.89 | 2 | ***Pik-s***, one unknown |
| | BN209 | - | 79 | 25 | 104 | - | 0.02 | - | 0.89 | 2 | ***Pib***, one unknown |
| | B90002 | 104 | 0 | 0 | 104 | - | - | - | - | | *Pia* |
| IR36 (VG3) | IK81-3 | - | 35 | 26 | 61 | 1.32 | - | - | 0.25 | 1 | ***Pita*** |
| | V850196 | - | 47 | 14 | 61 | - | 0.13 | - | 0.72 | 2 | ***Pita***, *Pik-s* |
| | BN209 | - | 45 | 16 | 61 | - | 0.05 | - | 0.82 | 2 | ***Pib***, one unknown |
| IR60 (VG3) | IK81-3 | - | 36 | 17 | 53 | - | 1.41 | - | 0.24 | 2 | ***Pita***, one uknown |
| | M64-1-3-9-1 | - | 40 | 13 | 53 | - | 0.02 | - | 0.89 | 2 | ***Pita***, *Piz-t* |
| | V850196 | - | 37 | 16 | 53 | - | 0.76 | - | 0.38 | 2 | ***Pita***, *Pik-s* |
| | BN209 | - | 48 | 5 | 53 | - | - | 0.46 | 0.5 | 3 | ***Pib***, two unknown |
| IR74 (VG5) | M36-1-3-10-1 | - | 36 | 40 | 76 | 0.22 | - | - | 0.64 | 1 | ***Pi20*(t)** |
| | BN111 | - | 51 | 25 | 76 | - | 2.52 | - | 0.11 | 2 | ***Pi20*(t), *Pik****[3)] |
| | PO6-6 | - | 34 | 42 | 76 | 0.84[b] | - | - | 0.36 | 1 | ***Pik**** |
| | Ca89 | - | 34 | 42 | 76 | 0.84[b] | - | - | 0.36 | 1 | ***Pik**** |
| | BN209 | - | 54 | 22 | 76 | - | 0.63 | - | 0.43 | 2 | ***Pik****, *Pib* |
| | B90002 | 76 | 0 | 0 | 76 | - | - | - | - | | *Pia* |
| IR46 (VG7a) | M36-1-3-10-1 | - | 58 | 42 | 100 | 2.56[d] | - | - | 0.11 | 1 | ***Pi20*(t)** |
| | BN111 | - | 58 | 42 | 100 | 2.56[d] | - | - | 0.11 | 1 | ***Pi20*(t)** |
| | IK81-25 | - | 59 | 41 | 100 | 3.24[c] | - | - | 0.07 | 1 | ***Pita*** |
| | M64-1-3-9-1 | - | 59 | 41 | 100 | 3.24[c] | - | - | 0.07 | 1 | ***Pita*** |
| | V850196 | - | 79 | 21 | 100 | - | 0.85 | - | 0.36 | 2 | ***Pita***, *Pik-s* |
| | BN209 | - | 81 | 19 | 100 | - | 1.92 | - | 0.17 | 2 | ***Pib***, one unknown |
| IR64 (VG7a) | BN111 | - | 37 | 35 | 72 | 0.06 | - | - | 0.81 | 1 | ***Pi20*(t)** |
| | IK81-25 | - | 41 | 31 | 72 | 1.39 | - | - | 0.24 | 1 | ***Pita*** |
| | M64-1-39-1 | - | 51 | 21 | 72 | - | 0.67 | - | 0.41 | 2 | ***Pita***, *Piz-t* |
| | BN209 | - | 52 | 17 | 69 | - | 0.08 | - | 0.78 | 2 | ***Pib***, one unknown |

1) Common letters (a, b, c, and d) indicate co-segregation among isolates.

2) Resistance genes described by bold letters were those estimated by the differential system (see Table 5).

3) *Pik**: One of the alleles *Pik*, *Pik-h*, *Pik-m*, or *Pik-p*.

The other genes segregating to BN111 and BN209 corresponded to *Pi20*(t) and *Pib*, respectively. In the analysis of segregation to B90002, all the lines were resistant, suggesting that IR74 carries the same *Pia* gene found in CO39. These results revealed that besides *Pita* and *Pik** confirmed in IR74, two other genes, *Pia* and *Pib* were identified in this variety.

Two varieties in VG 7a, IR46 and IR64, were analyzed to confirm the presence of *Pi20*(t), *Pita*, and *Pib*. In the analysis of IR46 involving 100 $BC_1F_2$ lines and four isolates, M36-1-3-10-1, BN111, IK81-25, and M64-1-3-9-1, a 1:1 ratio suggesting the monogenic segregation, and the co-segregation for resistance to BN111 and M36-1-3-10-1, and to IK81-25 and M64-1-3-9-1 were observed (Table 7). Since BN111 and M36-1-3-10-1 are avirulent to *Pi20*(t), and IK81-25 and M64-1-3-9-1 to *Pita*, the presence of *Pi20*(t)and *Pita* in IR46 was confirmed. A 3:1 ratio observed between segregating resistant and susceptible homozygote lines suggested that the segregation for resistance to isolates V850196 and BN209 was controlled by two dominant genes. In the reaction pattern of differential system, V850196 was avirulent to *Pita*, *Pik** and *Pik-s*, and BN209 to *Pib* and *Pik**. The gene *Pik** was estimated not to be harbored in VG 7a by reaction pattens. The other genes, *Pita* and *Pik-s*, were related to the segregation for resistance to V850196, and *Pib* and an unknown gene to BN209. Thus, *Pi20*(t), *Pita*, *Pik-s*, and *Pib*, and one unknown gene were identified in IR46.

## III. Allelism tests of the resistance genes using differential varieties

### Materials and methods

1. Rice plant materials

Nine IRRI rice varieties from 7 different VGs, IR34 (VG1b), IR24 (VG2a), IR36 and IR60 (VG3), PSBRc1 (VG4), IR74 (VG5), IR56 and IR70 (VG6), and IR64 (VG7b), were crossed with DVs with known blast resistance genes. The DVs were Toride 1 for *Piz-t*; BL 1 for *Pib*; Yashiromochi, C105TTP2L9, and C101PKT for *Pita*; Tsuyuake with *Pik-m* and Kanto 51 with *Pik* for *Pik**; and IR24 for three genes, *Pib*, *Pik-s*, and *Pi20*(t) (Imbe et al.1997). The $F_2$ populations derived from each cross were tested.

2. Inoculation and evaluation of resistance

The same procedure described above was employed for blast inoculation test. The $F_2$ populations were inoculated with selected blast isolates, which were avirulent to particular genes.

3. Identification of resistance gene

The allelic relationship of the genes was determined based on the segregation for resistance to a particular blast isolate in the $F_2$ populations. If the segregation did not include susceptible plants, it was assumed that both parents had the same or an allelic gene (Fig. 7). Otherwise, it was considered that the resistance was governed by different allele genes (Fig. 8).

Same allele case

Test variety $(A^TA^T)$ × Differential variety Y $(A^DA^D)$

$F_1$ $(A^TA^D)$

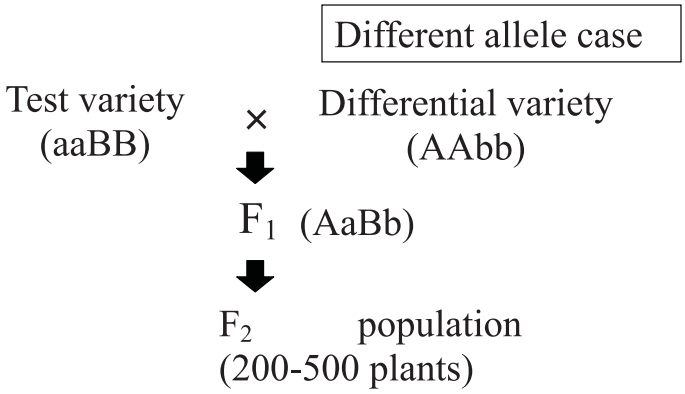
$F_2$ population (200-500 plants)

| ♂ \ ♀ | $A^T$ | $A^D$ |
|---|---|---|
| $A^T$ | $A^TA^T$ | $A^DA^T$ |
| $A^D$ | $A^TA^D$ | $A^DA^D$ |

Phenotype; All resistant plants

**Appendix 7.** Allelism test using an $F_2$ population derived from a cross between a test rice variety and differential varieties – a case of the same allele

AT, AD; resistance gene in the rice test variety and differential variety, respectively.
Inoculation test is carried out with a blast isolate avirulent to the differential variety.




| ♂ \ ♀ | AB | Ab | aB | ab |
|---|---|---|---|---|
| AB | **AABB** | **AABb** | **AaBB** | **AaBb** |
| Ab | **AABb** | **AAbb** | **AaBb** | **Aabb** |
| aB | **AaBB** | **AaBb** | aaBB | aaBb |
| ab | **AaBb** | **Aabb** | aaBb | aabb |

•Segregation ratio in case of inoculation with a blast isolate avirulent only to the differential variety.
→ Resistance plants: Susceptible plants = 3 : 1
•Segregation ratio in case of inoculation with a blast isolate avirulent to both the differential and the test variety.
→ Resistance plants : Susceptible plants = 15 : 1

**Appendix 8.** Allelism test using $F_2$ population derived from the cross between rice test variety and differential variety – a case of different allele

A, B; resistance gene in the differential and the test variety, respectively.
A blast isolate avirulent to the differential variety carrying the known resistance gene is used for the inoculation test.

**Results and discussion**

1. Gene identification in IR64

The $F_2$ populations were generated from crosses between IR64 of VG7a and 5 DVs, IR24 for *Pi20*(t), *Pik-s* and *Pib*, C105TTP2L9 and C101PKT for *Pita*, Toride 1 for *Piz-t*, and IR56 for *Pita* and *Pik**. When the $F_2$ populations derived from cross between IR64 and IR24 were tested against 3 blast isolates, BN111 avirulent to *Pi20*(t) and *Pik*, V850196 avirulent to *Pita*, *Pik* and *Pik-s*, and BN209 avirulent to *Pik* and *Pib*, no population shows any susceptible plants to these isolates (Table 8). These results indicated that IR64 was revealed to harbor the same alleles of *Pi20*(t), *Pik-s* and *Pib* in IR24 by inoculation test against BN111, V850196 and BN209, respectively. The $F_2$ plants derived from crosses between IR64 and 2 DVs for *Pita*, C105TTP2L9 and C101PKT were all resistant to a blast isolate, IK81-25 avirulent to *Pita*. This suggested that IR64 also harbored the same allele of *Pita* in those DVs. Similarly, *Piz-t* was identified in IR64, when the $F_2$ plants derived from cross between IR64 and Toride 1 which is a DV for *Piz-t* were all resistant to both M39-1-3-8-1 and M64-1-3-9-1 which are avirulent to the gene. In the $F_2$ population derived from a cross between IR64 and IR56, all tested plants were resistant to IK81-25 avirulent to *Pita*. On the other hand, a 3:1 segregation ratio ($\chi^2$= 0.22, $p$=0.64) was observed in the same population for resistance to PO6-6, which is avirulent to *Pik**. This resistance was suggested to be governed by a single gene, *Pik** in IR56. These results confirmed that IR64 harbored 6 resistance genes, *Pi20*(t), *Pita*, *Pib*, *Pik-s*, *Piz-t*, and one unknown.

2. Gene identification in IRRI-bred varieties

To confirm the presence of *Piz-t* and *Pib* in IR34 in VG1b, $F_2$ populations derived from crosses with 2 DVs, Toride 1 for *Piz-t* and BL 1 for *Pib* were tested against the selected blast isolates. The $F_2$ population derived from cross with Toride 1 was inoculated with 2 blast isolates, M64-1-3-9-1 and M39-1-3-8-1 both avirulent to Piz-t (Table 9). As a result, all plant of the population was resistant to these isolate. Thus, the resistance gene in IR34 to these isolate was the same as that in Toride 1, *Piz-t*. In the same manner, $F_2$ population derived from cross with BL1 did not show any susceptible plants to blast isolate BN209 avirulent to *Pib*. These results suggested the presence of *Piz-t* and *Pib* in IR34.

All $F_2$ plants derived from the cross between IR24 in VG2a and a DV, BL 1 for *Pib* was resistant to BN209

avirulent to *Pik** and *Pib* (Table 9). These results suggested the presence of *Pib* in IR24 and supported the previous report by Imbe et al. (1997).

Four kind of $F_2$ population were generated from crosses between IR36 in VG3 and 4 DVs; C105TTP2L9 and C101PKT for *Pita*, Toride 1 for *Piz-t*, and BL 1 for *Pib*. When these populations were tested against 4 isolates, IK81-25 (avirulent to *Pita*), M391-3-8-1 and M64-4-1-3-9-1 (avirulent to *Pita* and *Piz-t*), and BN209 (avirulent to *Pib*), all $F_2$ plants showed resistance. These results confirmed the presence of *Pita*, *Piz-t*, and *Pib* in IR36. The presence of *Piz-t* was also confirmed by the allelism test between IR34 and IR36 using M39-1-3-8-1 and M64-1-3-9-1. Using $F_2$ population derived from a cross between IR36 and IR24, the allelism of *Pib* and *Pik-s* was confirmed by inoculation test against blast isolate BN209 (avirulent to *Pib*) and V850196 (avirulent to *Pik-s*), respectively. The reaction of *Piz-t* was masked by *Pita* and could not be estimated in the previous analysis based on reaction pattern. However, *Piz-t* was identified in IR36 through allelism tests. Thus, $BC_1F_2$ analysis and allelism tests revealed that IR36 carries at least four genes— *Pita*, *Pik-s*, *Pib*, and *Piz-t*.

The $F_2$ populations derived from crosses of IR60 in VG3 with IR34 and IR24 did not segregate any susceptible plants to 4 isolates, BN209 (avirulent to *Pib* and *Pik**), V850196 (avirulent to *Pita*, *Pik-s* and *Pik**), M39-1-3-8-1 and M64-1-3-9-1 (avilurent to *Pita* and *Piz-t*) (Table 9). IR34 was confirmed to harbor *Pia*, *Pib*, *Piz-t*, and *Pik-s* in this study. IR24 was also confirmed to harbor *Pia*, *Pib*, *Pik-s*, and *Pi20*(t), previously. IR60 was estimated not to harbor *Pik** by reaction pattern, while the resistance to M39-1-3-8-1 and M64-1-3-9-1 was governed by *Piz-t* which was estimated also in IR34. From these results, IR60 was revealed to have 3 genes, *Pib*, *Piz-t*, and *Pik-s*.

PSBRc1 of VG4 was crossed with IR56, which was estimated to be carrying *Pita* and *Pik** to generate $F_2$ population. All of the $F_2$ plants was resistant to isolates PO6-6 avirulent to *Pik** and BN111 avirulent to both *Pik** and *Pi20*(t) (Table 9). Since *Pi20* was not estimated in VG4 by reaction patterns in the previous analysis, the resistance of $F_2$ plants to these isolates suggested the allelism of *Pik** in PSBRc1 and IR56. The presence of *Pita* was also verified in this population using IK81-25 which is avirulent to *Pita*. Results showed that the $F_2$ plants segregated into 406 resistant and 126 susceptible, which fit the 3:1 ratio ($\chi^2$=0.51, p=0.43). This indicated that a single dominant gene, *Pita* previously estimated in IR56 governed the segregation, because PSBRc1 was estimated not to have *Pita* by reaction pattern.

**Table 8.** Allelism test for resistance using $F_2$ populations derived from the crosses between IR64 and differential varieties

| Cross combination (Resistance gene harbored in parent) | | Differntial blast isolate used for the seregation analysis of resistance | Reaction of monogenic line | | | | | | No. of plants[1)] | | | $\chi^2$ value (3:1) | *P* (df =1) | Identified gene |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | IRBL20-IR24 (*Pi20*) | IRBLta-K1 (*Pita*) | IRBLk-Ka (*Pik*) | IRBLb-B (*Pib*) | IRBLzt-T (*Piz-t*) | IRBLks-F5 (*Pik-s*) | R | S | Total | | | |
| IR24 (*Pib*, *Pi20*(t), *Pik-s*) | / IR64 (*Pi20*(t), *Pita*, *Pib*) | BN111 | R | S | R | S | S | S | 302 | 0 | 302 | - | - | *Pi20(t)* |
| | | V850196 | S | R | R | S | S | R | 227 | 0 | 227 | - | - | *Pik-s* |
| | | BN209 | S | S | R | R-M | S | S | 222 | 0 | 222 | - | - | *Pib* |
| IR64 | / C105TTP2L9 (*Pita*) | IK81-25 | S | R | S | S | S | S | 274 | 0 | 274 | - | - | *Pita* |
| IR64 | / C101PKT (*Pita*) | IK81-25 | S | R | S | S | S | S | 132 | 0 | 132 | - | - | *Pita* |
| IR56 (*Pita*, *Pik**) | / IR64 | IK81-25 | S | R | S | S | S | S | 296 | 0 | 296 | - | - | *Pita* |
| | | PO6-6 | S | S | R | S | S | S | 193 | 60 | 253 | 0.22 | 0.64 | *Pik** in IR56 |
| Toride1 (*Piz-t*) | / IR64 | M39-1-3-8-1 | S | R | S | S | R | S | 124 | 0 | 124 | - | - | *Piz-t* |
| | | M64-1-3-9-1 | S | R | S | S | R | S | 133 | 0 | 133 | - | - | *Piz-t* |

1) R; resistant, S; susceptible.

2) *Pik**: One of the alleles *Pik*, *Pik-h*, *Pik-m*, or *Pik-p*.

IR24 was expected not to habor Pita based on the reaction pattern to differential blast isolates in previous reasearch.

IR24 and IR64 were expected not to harbor Pik based on the reaction patterns to differential blast isolates in previous reasearch.

Toride 1 dose not harbor *Pita*

**Table 9.** Allelism test for resistance using $F_2$ populations derived from the crosses between IRRI-bred and differential varieties

| Variety (VG) | Cross combination (Resistance gene(s) harbored in parent) | | Isolate | No. of plants[1)] | | | $\chi^2$ value (3:1) | *P* (df = 1) | Identified gene |
|---|---|---|---|---|---|---|---|---|---|
| | | | | R | S | Total | | | |
| IR34 | Toride1 (*Piz-t*) | / IR34 (*Pia*, *Pik-s*, *Pib*, *Piz-t*) | M39-1-3-8-1 | 94 | 0 | 94 | - | - | *Piz-t* |
| (VG1b) | | | M64-1-3-9-1 | 84 | 0 | 84 | - | - | *Piz-t* |
| | BL1 (*Pib*) | / IR34 | BN209 | 437 | 0 | 437 | - | - | *Pib* |
| IR24 (VG2b) | BL1 | / IR24 | BN209 | 161 | 0 | 161 | - | - | *Pib* |
| IR36 | IR36 (P*ita*, *Pib*, *Pik-s*) | / C105TTP2L9 (*Pita*) | IK81-25 | 468 | 0 | 468 | - | - | *Pita* |
| (VG3) | IR36 | / C101PKT (*Pita*) | IK81-25 | 167 | 0 | 167 | - | - | *Pita* |
| | BL1 | / IR36 | BN209 | 420 | 0 | 420 | - | - | *Pib* |
| | IR24 | / IR36 | V850196 | 295 | 0 | 295 | - | - | *Pik-s* |
| | | | BN209 | 287 | 0 | 287 | - | - | *Pib* |
| | Toride 1 | / IR36 | M39-1-3-8-1 | 184 | 0 | 184 | - | - | *Piz-t* |
| | | | M64-1-3-9-1 | 168 | 0 | 168 | - | - | *Piz-t* |
| | IR34 | / IR36 | M39-1-3-8-1 | 467 | 0 | 467 | - | - | *Piz-t* |
| | | | M64-1-3-9-1 | 516 | 0 | 516 | - | - | *Piz-t* |
| IR60 | IR34 | / IR60 (*Pita*, *Pib*, *Pik-s*, *Piz-t*) | M39-1-3-8-1 | 302 | 0 | 302 | - | - | *Piz-t* |
| (VG3) | | | M64-1-3-9-1 | 303 | 0 | 303 | - | - | *Piz-t* |
| | IR24 | / IR60 | V850196 | 235 | 0 | 235 | - | - | *Pik-s* |
| | | | BN209 | 360 | 0 | 360 | - | - | *Pib* |
| PSBRc1 | IR56 (*Pita*, *Pik**) | / PSBRc1 (*Pik**[2)]) | PO6-6 | 531 | 0 | 531 | - | - | *Pik** |
| (VG4) | | | BN111 | 526 | 0 | 526 | - | - | *Pik** |
| | | | IK81-25 | 406 | 126 | 532 | 0.51 | 0.43 | *Pita* in IR56 |
| IR74 | IR56 | / IR74 (*Pi20*(t), *Pik**) | PO6-6 | 560 | 0 | 560 | - | - | *Pik** |
| (VG5) | | | BN111 | 585 | 0 | 585 | - | - | *Pik** |
| | | | IK81-25 | 442 | 135 | 577 | 0.82 | 0.37 | *Pita* in IR56 |
| IR56 | C101PKT | / IR56 | IK81-25 | 469 | 0 | 469 | - | - | *Pita* |
| (VG6) | | | PO6-6 | 358 | 85 | 443 | 7.98 | 0.005 | *Pik** in IR56 |
| | Yashiromochi (*Pita*) | / IR56 | IK81-25 | 463 | 0 | 463 | - | - | *Pita* |
| | | | PO6-6 | 370 | 127 | 497 | 0.08 | 0.78 | *Pik** in IR56 |
| | IR56 | / Kanto 51 (*Pik*) | PO6-6 | 440 | 0 | 440 | - | - | *Pik** |
| | | | BN111 | 407 | 0 | 407 | - | - | *Pik** |
| | IR56 | / Tsuyuake (*Pik-m*) | PO6-6 | 498 | 0 | 498 | - | - | *Pik** |
| | | | BN111 | 496 | 0 | 496 | - | - | *Pik** |
| IR70 | IR56 | / IR70 (*Pita*, *Pik**) | IK81-25 | 567 | 0 | 567 | - | - | *Pita* |
| (VG6) | | | PO6-6 | 577 | 0 | 577 | - | - | *Pik** |
| | | | BN111 | 558 | 0 | 558 | - | - | *Pik** |

1) R; resistant, S; susceptible.
2) *Pik**; One of the alleles *Pik*, *Pik-h*, *Pik-m*, or *Pik-p*.

*Pik** was identified in IR74 by an allelism test with IR56 (Table 9). All the $F_2$ plants derived from a cross of IR74 with IR56 were resistant to PO6-6, which is avirulent to *Pik**, and BN111 avirulent to both *Pik** and *Pi20*(t), suggesting that IR74 had the same allele of *Pik** in IR56. The segregation ratio for resistance to IK81-25, which was avirulent to *Pita* fitted to a 3:1 ratio ($\chi^2$= 0.82, p=0.37) expected for a single dominant gene control. This was thought to be due to *Pita* in IR56 but not in IR74.

In VG6, two genes, *Pita* and *Pik**, were previously estimated by reaction patterns in IR56 and IR70. To confirm their presence in these varieties, IR56 was crossed with 4 DVs, C101PKT and Yashiromochi for *Pita*, Kanto 51 and Tsuyuake for *Pik**, and with IR70 for *Pita* and *Pik**. On these 5 kinds of $F_2$ populations, 11 combinations of allelism test were carried out using 3 isolates: IK81-25 avirulent to *Pita*, PO6-6 to *Pik**, and BN111 to *Pik** and *Pi20*(t). Of these, two $F_2$ populations derived from crosses of IR56 with C101PKT and Yashiromochi showed a single gene segregation to PO6-6, suggesting that the segregation was due to *Pik** in IR56 (Table 9). The other 9 combinations did not segregate any susceptible plants, suggesting that the same gene in IR56 or IR70 and DVs controlled the resistance. Since blast isolates from the Philippines could not differentiate the *Pik* alleles, except *Pik-s*, no susceptible plants were obtained from the populations derived from the combinations among DVs, Kanto 51, Tsuyuake, and IR56 for *Pik**. These results confirmed that IR56 and IR70 harbored Pita and *Pik**.

**Conclusions**

A total of 42 IRRI-bred rice varieties were classified into seven variety groups and estimated at least seven blast resistance genes, *Pi20*(t), *Pita*, *Pik**, *Pib*, *Pik-s*, *Piz-t*, and *Pii* or *Pi3*(t), following a differential system using Philippine isolates of *P. grisea* (Ebron et al., 2004, Fukuta et al. 2007). In the present study, the presence of seven blast resistance genes, *Pi20*(t), *Pita*, *Pik**, *Pia*, *Pib*, *Pik-s*, and *Piz-t*, was confirmed through genetic analyses in 10 varieties: IR34 (VG1b), IR24 (VG2a), IR36 and IR60 (VG3), PSBRc1 (VG4), IR74 (VG5), IR56 and IR70 (VG6), and IR46 and IR64 (VG7a) (Table 5, 7, and 9). Four of these genes, *Pia*, *Pib*, *Pik-s*, and *Piz-t*, were identified also in some varieties. Two genes *Pib* and *Pik-s* or *Pik** were previously estimated in nearly all the varieties and their presence confirmed in the present study. The genes *Pita*, *Pik* alleles (*Pik** and *Pik-s*), *Piz-t*, and *Pib* were identified frequently in Indica-type varieties (Kiyosawa, 1966; Kiyosawa and Murty, 1969; Yokoo and Kiyosawa, 1970; Yokoo et al., 1978). Our results agreed with the findings of these previous studies.

The present study reports the genotypes of blast resistance in IRRI-bred rice varieties determined by segregation analyses using backcrossed progenies and allelism tests with DVs to confirm the estimated resistance genes and to postulate the masked ones in the IRRI-bred varieties' genetic backgrounds. The utility of genetic analyses based on the differential system was demonstrated in this study, and this information will be very useful whenever IRRI-bred varieties are used in any rice breeding program. Genetic analyses based on the differential system are useful tools for the identification of resistance genes, although these are complicated and relatively tedious. Over the past several years, two genes *Pita* and *Pib* have already been isolated and their detailed sequences described by Wang et al. (1999) and Jia et al. (2002), respectively. Based on this information, molecular markers specific to both genes were developed and can be used to identify the genotypes of *Pib* and *Pita* in Indica-type varieties. Therefore, Indica-type, IRRI-bred varieties, which harbor several resistance genes and show complex reactions, can be determined of these genotypes using molecular markers that can complement detection of the genes by standard genetic analysis.

The differential system, which is based on conventional methods and which does not require advanced facilities, was revealed to be useful as a fundamental tool to provide essential information to develop breeding programs for blast resistance.

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